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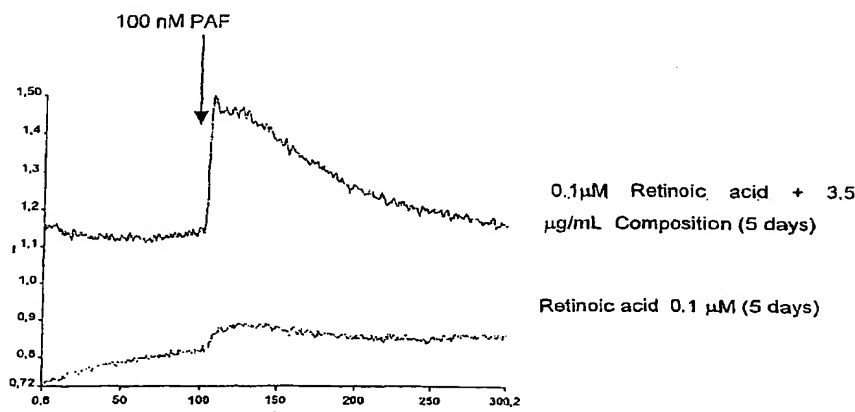
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(54) Title: COMPOSITION OF LABDANE DITERPENES EXTRACTED FROM *ANDROGRAPHIS PANICULATA*, USEFUL FOR THE TREATMENT OF AUTOIMMUNE DISEASES, AND ALZHEIMER DISEASE BY ACTIVATION OF PPAR-GAMMA RECEPTORS



(57) Abstract: Composition characterized for being constituted of molecules identified as diterpenic labdanes that are obtained from a valuable plant species from *Andrographis paniculata*, dried herb by a special process of extraction, for the treatment of autoimmune other immunological diseases in human's beings and animals, exhibiting a low toxicity and absence of significant side effects. Likewise, the new composition is useful for preparing a pharmaceutical composition useful for preventing and treating Alzheimer's disease. The composition inhibits the synthesis of pro-inflammatory cytokines, as a result of activating the PPAR gamma receptor and diminishing the nuclear factor kappa B. In addition, to its own therapeutic features, this new composition solves the inconveniences of the current products, medicines and procedures, that have an important toxicity level and/or cause non desired side effects. The present invention has applications in immunopharmacology, clinical and pre clinical human and veterinary pharmacology, pharmaceutical technology, rheumatology, and clinical immunology, immunotherapy, organ and tissue transplantation, and the immunodeficiency diseases as AIDS.

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**COMPOSITION OF LABDANE DITERPENES EXTRACTED FROM  
*ANDROGRAPHIS PANICULATA*, USEFUL FOR THE TREATMENT OF  
AUTOIMMUNE DISEASES, AND ALZHEIMER DISEASE BY  
ACTIVATION OF PPR-GAMMA RECEPTORS**

**DESCRIPTION**

The main objective of immunopharmacology and biopharmacy, is the continuous search of new therapeutic solutions for treating the symptoms and modify the course of immunological diseases.

**BACKGROUND OF THE INVENTION**

Autoimmune diseases are characterized by a spontaneous reaction of the immune system against its own organism. These reactions are caused by the recognition of auto-antigens by the T lymphocytes, which are responsible to trigger humoral (auto-antibodies production) and cellular (increased lymphocytes and macrophages cytotoxic activity) immune responses. Autoimmune diseases include: rheumatoid diseases, psoriasis, systemic dermatomyocytis, multiple sclerosis, lupus erythematosus or exacerbated immune responses by antigens, i.e. asthma, allergies to drugs and food, etc. All these diseases are limiting, chronic, and in some cases lethal, and no effective therapy exists nowadays to treat them. Therefore, any drug, medicine, or media that is able to cause remission or decrease in the course of the disease, represents a significant solution for the patients health.

The search for a treatment for autoimmune diseases has resulted in an important effort to find suitable drugs and methods.

At present, the treatment of these diseases is principally based in the use of immunosuppressant drugs, such as glucocorticoids, calcineurin inhibitors, and

antiproliferatives-antimetabolites. However, since these pharmacological therapies act in many different targets, they can reduce the immune function as a whole, or due to long term use can have the disadvantage of different cytotoxic effects, and therefore can suppress the immune system in a non specific way, exposing the patient to the risk of infections and cancer. Calcineurin and glucocorticoids exhibit an additional disadvantage, due to their nephrotoxicity and diabetogenic effects, that limits their utility in several clinical conditions (e.g. renal insufficiency, diabetes).

The latest therapeutic advances in immuno-suppression are the anti CD3 monoclonal antibodies; the anti IL-2 receptor monoclonal antibodies and the anti-TNF $\alpha$  monoclonal antibodies. Despite the fact that these treatments exhibit marked immunosuppressing effects, anaphylaxis reactions, opportunistic infections (Tuberculosis) and neoplasm's, fever, urticaria, hypotension, dyspnea are associated with these medicines, representing a serious problem in the application of said compositions and pharmaceutical products. In injectable applications, one out of three patient can present itching, swelling, and pain.

## BRIEF SUMMARY OF THE INVENTION

The composition claimed in the present invention, is able to diminish the immune response, which characterizes autoimmune diseases, allergies, alleviating the symptoms and the course of the disease, with maintenance of the "immunological tolerance".

In other words, the composition disclosed in the present invention, is essentially characterized according to the immunological tolerance originated by it, which corresponds to the active state of the absence of a specific reaction against an antigen, without causing the side effects of the current immunosuppressant drugs.

Specifically, this composition inhibits the synthesis and expression of interferon gamma, IL-2 by stimulating the PPAR gamma receptor and reducing the NF kappaB factor.

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Consequently, this new composition of diterpenic labdanes is characterized by selectively reducing the cytokines over-expression, which is involved in the pathogenesis of autoimmune diseases.

10 Recent advances in scientific understanding of the mediators involved in acute and chronic inflammatory diseases and cancer have led to new strategies in the search for effective therapeutics. Traditional approaches include direct target intervention such as the use of specific antibodies, receptor antagonists, or enzyme inhibitors having all of them an important level of side effects (e.g. allergies, gastrointestinal  
15 ulcers, bleedings, etc.). Recent breakthroughs in the elucidation of regulatory mechanisms involved in the transcription and translation of a variety of mediators have led to increased interest in therapeutic approaches directed at the level of gene transcription (e.g. COX2, iNOS, IL1beta, TNFalpha, ICAM, etc.).

20 One of the most important mediators is NF-kappaB that belongs to a family of closely related dimeric transcription factor complexes composed of various combinations of the Rel/NF-kappaB family of polypeptides. The family consists of five individual gene products in mammals, RelA (p65), NF-kappaB1 (p50/p105), NF-kappaB2 (p49/p100), c-Rel, and RelB, all of which can form hetero- or  
25 homodimers. These proteins share a highly homologous 300 amino acid "Rel homology domain" which contains the DNA binding and dimerization domains. At the extreme C-terminus of the Rel homology domain is a nuclear translocation sequence important in the transport of NF-kappaB from the cytoplasm to the

nucleus. In addition, p65 and cRel possess potent trans-activation domains at their C-terminal ends.

The activity of NF-kappaB is regulated by its interaction with a member of the inhibitor IkappaB family of proteins. This interaction effectively blocks the nuclear localization sequence on the NF-kappaB proteins, thus preventing migration of the dimer to the nucleus. A wide variety of stimuli activate NF-kappaB through what are likely to be multiple signal transduction pathways. Included are bacterial products (LPS), some viruses (HIV-1, HTLV-1), inflammatory cytokines (TNFalpha, IL-1), and environmental stress. Apparently common to all stimuli however, is the phosphorylation and subsequent degradation of IkappaB. IkappaB is phosphorylated on two N-terminal serines by the recently identified IkappaB kinases (*IKK*-alpha and *IKK*-beta). Site-directed mutagenesis studies indicate that these phosphorylations are critical for the subsequent activation of NF-kappaB in that once phosphorylated the protein is flagged for degradation via the ubiquitin-proteasome pathway. Free from IkappaB, the active NF-kappaB complexes are able to translocate to the nucleus where they bind in a selective manner to preferred gene-specific enhancer sequences. Included in the genes regulated by NF-kappaB are a number of cytokines, cell adhesion molecules, and acute phase proteins.

It is well-known that NF-kappaB plays a key role in the regulated expression of a large number of pro-inflammatory mediators including cytokines such as IL-6 and IL-8. Cell adhesion molecules, such as ICAM and VCAM, and inducible nitric oxide synthase (iNOS). Such mediators are known to play a role in the recruitment of leukocytes at sites of inflammation and in the case of iNOS, may lead to organ destruction in some inflammatory and autoimmune diseases.

The importance of NF-kappaB in inflammatory disorders is further strengthened by studies of airway inflammation including asthma, in which NF-kappaB has been shown to be activated. This activation may underlie the increased cytokine production and leukocyte infiltration characteristic of these disorders. In addition, inhaled steroids are known to reduce airway hyperresponsiveness and suppress the inflammatory response in asthmatic airways. In light of the recent findings with regard to glucocorticoid inhibition of NFkappaB, one may speculate that these effects are mediated through an inhibition of NFkappaB.

Further evidence for a role of NF-kappaB in inflammatory disorders comes from studies of rheumatoid synovium. Although NF-kappaB is normally present as an inactive cytoplasmic complex, recent immunohistochemical studies have indicated that NF-kappaB is present in the nuclei, and hence active, in the cells comprising rheumatoid synovium. Furthermore, NF-kappaB has been shown to be activated in human synovial cells in response to stimulation with TNF-alpha. Such a distribution may be the underlying mechanism for the increased cytokine and eicosanoid production characteristic of this tissue. See Roshak, A. K., et al., J. Biol. Chem., 271, 31496-31501 (1996).

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The NF-kappaB/Rel and IkappaB proteins are also likely to play a key role in neoplastic transformation. Family members are associated with cell transformation *in vitro* and *in vivo* as a result of over-expression, gene amplification, gene rearrangements or translocations. In addition, rearrangement and/or amplification of the genes encoding these proteins are seen in 20-25% of certain human lymphoid tumors. In addition, a role for NF-kappaB in the regulation of apoptosis has been reported strengthening the role of this transcription factor in the control of cell proliferation.

10 The first plant-derived modulators of NF-kB were reported nearly a decade ago by Kopp & Ghosh (1994) who identified sodium salicylate and its semi-synthetic derivative, aspirin. Following this discovery, a number of new natural products, of different chemical classes, have demonstrated NF-kB inhibitory activity.

15 Several NF-kappaB inhibitors are described in the literature C. Wahl, et al. J. Clin. Invest. 101(5), 1163-1174 (1998), R. W. Sullivan, et al. J. Med. Chem. 41, 413-419 (1998), J. W. Pierce, et al. J. Biol. Chem. 272, 21096-21103 (1997). The marine natural product hymenialdisine is known to inhibit NF-kappaB. Roshak, A., et al., JPET, 283, 955-961 (1997). Breton, J. J and Chabot-Fletcher, M. C., JPET, 282, 459-466 (1997). Salicylanilides are known compounds and described  
20 by M. T. Clark, R. A. Coburn, R. T. Evans, R. J. Genco, J. Med. Chem., 1986, 29, 25-29.

Recently, an important mechanism of the inhibition of NF-kappaB suggest the possible activation of the receptors for peroxisomes.

25

The receptors for peroxysomes known as "Peroxisomes Proliferator Activated Receptors" (PPARs), [attending to the usual use of the term, expressed in its abbreviation known in the scientific area, said abbreviation shall be used for identifying this receptor] have been implicated in autoimmune diseases and other

diseases, i.e diabetes mellitus, cardiovascular and gastrointestinal disease, and Alzheimer's disease. The current pharmaceutical agents with PPAR gamma agonist are still in the experimental stage and have significant side effects for human health, due to its mechanism of action. Therefore, there is the need to develop new agents with lesser toxic effects that can modulate these receptors more accurately in order to prevent, treat and/or alleviate the above mentioned diseases or conditions.

This new composition, modulates these receptors more accurately, and therefore allows preventing, treating and/or alleviating autoimmune diseases more efficiently, without causing undesirable side effects to the patients.

Peroxisomes Proliferator Activated Receptors (PPARs) are members of the nuclear hormone receptor super family, which are ligand-activated transcription factors regulating gene expression. Various subtypes of PPARs have been discovered. These include PPARalpha, PPARbeta or NUC1, PPAR $\gamma$  and PPARdelta.

PPAR $\gamma$  was characterized originally as a key regulator of adipocyte differentiation and lipid metabolism. PPAR $\gamma$  expression is directed by different promoters, leading to three PPAR $\gamma$  isoforms. It is now clear that PPAR $\gamma$  is also found in other cell types including fibroblasts, myocytes, breast cells, the white and red pulp of rat spleen, human bone-marrow precursors, and macrophages/monocytes. In addition, PPAR $\gamma$  has been shown in macrophage foam cells in atherosclerotic plaques. An important role for PPAR $\gamma$  in glucose metabolism was identified when it was demonstrated that a class of antidiabetic drugs, the thiazolidinediones, were high-affinity PPAR $\gamma$  ligands. The thiazolidinediones were developed originally for the treatment of type-2 diabetes on the basis of their ability to lower glucose levels (and levels of circulating fatty acids) in rodent models of insulin resistance. The

finding that the thiazolidinediones mediate their therapeutic effects through direct interactions with PPAR $\gamma$  established PPAR $\gamma$  as a key regulator of glucose and lipid homeostasis. Despite being described initially as a regulator of lipid and glucose metabolism, PPAR $\gamma$  has also been demonstrated recently to have a role in cell proliferation and malignancy. Ligands for PPAR $\gamma$  have been shown to mediate positive and negative effects on cell proliferation and malignancy.

In addition to the thiazolidinedione class of antidiabetic drugs, a variety of nonsteroidal anti-inflammatory drugs also can function as PPAR $\gamma$  ligands, although the latter have relatively low affinity.

The prostaglandin D<sub>2</sub> (PGD<sub>2</sub>) dehydration product PGJ<sub>2</sub> was the first endogenous ligand discovered for PPAR $\gamma$ . The additional PGD<sub>2</sub> dehydration product, 15-deoxy- $\Delta^{12,14}$ -PGJ<sub>2</sub> (15d-PGJ<sub>2</sub>), is also a naturally occurring substance that binds directly to PPAR $\gamma$  and is a potent ligand for PPAR $\gamma$  activation.

One of the earliest findings associating PPARs and macrophages was that PPAR $\gamma$  was highly expressed in macrophage-derived foam cells of human and murine atherosclerotic lesions. Subsequently, it has been demonstrated that PPAR $\gamma$  is expressed in human and murine monocytes/macrophages. Functionally, PPAR $\gamma$  has been shown to play a role in the differentiation and activation of monocytes and in the regulation of inflammatory activities.

Many studies have demonstrated that PPAR $\gamma$  ligands inhibit macrophage-inflammatory responses. The anti-inflammatory effects of PPAR $\gamma$  activation have been demonstrated with human and murine monocyte/macrophages and monocyte/macrophage lines. Activation of macrophages normally leads to the secretion of several different proinflammatory mediators. Treatment with 15d-PGJ<sub>2</sub>

or thiazolidinediones has been found to inhibit the secretion of many of these mediators (including gelatinase B, IL-6, TNF- $\alpha$ , and IL-1 $\beta$ ) and also to reduce the induced expression of inducible NOS (iNOS) and the transcription of the scavenger receptor-A gene.

5

The relevance of PPAR $\gamma$  has been studied in several human autoimmune diseases and animal models of autoimmune diseases. Kawahito et al. demonstrated that synovial tissue expressed PPAR $\gamma$  in patients with rheumatoid arthritis (RA).

PPAR $\gamma$  was found to be highly expressed in macrophages, and modest expression was noted in synovial-lining fibroblasts and ECs. Activation of PPAR $\gamma$  by 15d-PGJ<sub>2</sub> and troglitazone induced RA synoviocyte apoptosis *in vitro*.

It has been suggested that PPAR $\gamma$  is functionally relevant in freshly isolated T cells or becomes functionally relevant early in activation. In these studies, it was also demonstrated that the two ligands for PPAR $\gamma$  mediated inhibition of IL-2 secretion by the T-cell clones and did not inhibit IL-2-induced proliferation of such clones.

Several studies have investigated the role of PPAR $\gamma$  ligands in modifying animal models of autoimmune diseases. Su et al. showed that in a mouse model of inflammatory bowel disease, thiazolidinediones markedly reduced colonic inflammation. It has been proposed that this effect might be a result of a direct effect on colonic epithelial cells, which express high levels of PPAR $\gamma$  and can produce inflammatory cytokines. Kawahito et al. demonstrated that intraperitoneal administration of the PPAR ligands, 15d-PGJ<sub>2</sub> and troglitazone, ameliorated adjuvant-induced arthritis. Nino et al. examined the effect of a thiazolidinedione on experimental allergic encephalomyelitis and found that this treatment attenuated the inflammation and decreased the clinical symptoms in this mouse model of multiple sclerosis. Finally, Reilly et al. demonstrated that renal glomerular

mesangial cells are important modulators of the inflammatory response in lupus nephritis, secreting, when activated, inflammatory mediators including NO and cyclooxygenase products, thus perpetuating the local inflammatory response.

Given the above studies, the relevance of PPARs and the utility of treatment with PPAR agonists in diseases with an inflammatory or autoimmune pathogenesis will likely continue to remain a research focus.

Recently, the issue of the specificity of 15d-PGJ<sub>2</sub> for PPAR $\gamma$  has been at least partially clarified. NF- $\kappa$ B is a critical activator of genes involved in inflammation and immunity. In this activation, the I $\kappa$ B kinase complex (IKK) phosphorylates the NF- $\kappa$ B inhibitors (I $\kappa$ B proteins) leading to their conjugation with ubiquitin and subsequent degradation by proteosome. This then allows freed NF- $\kappa$ B dimers to translocate to the nucleus and induce target genes. Rossi et al. demonstrated that the cyclopentenone PGs, including 15d-PGJ<sub>2</sub>, directly inhibit and modify the IKK2 subunit of IKK. This, in turn, prevents the phosphorylation of the inhibitory I $\kappa$ B proteins that then target these proteins for ubiquitin conjugation and degradation. This then prevents the activation of NF- $\kappa$ B. Similarly, Castrillo et al. showed that in RAW 264.7 macrophage cells treated with LPS and IFN- $\alpha$ , incubation with 15d-PGJ<sub>2</sub> resulted in a significant inhibition of IKK2 activity and an inhibition of the degradation of the inhibitory I $\kappa$ B proteins. This, in turn, caused a partial inhibition of NF- $\kappa$ B activity and an impaired expression of genes requiring NF- $\kappa$ B activation, such as type-2 NOS and cyclooxygenase 2.

Therefore, it can be concluded that PPAR $\gamma$  and NF- $\kappa$ B are important mediators involved in autoimmune diseases, resulting in a stimulus to the pharmaceutical industry to search for new selective drugs and medicines that affects these mediators.

On the other hand, Alzheimer's disease (AD) is characterized by the extracellular deposition of  $\beta$ -amyloid fibrils within the brain and the activation of microglial cells associated with the amyloid plaque. The activated microglia subsequently secretes a diverse range of inflammatory products. Kitamura et al. assessed the occurrence of PPAR $\gamma$  and COX-1, COX-2, in normal and AD brains using specific antibodies and found increased expression of these moieties in AD brains. Nonsteroidal, anti-inflammatory drugs (NSAIDs) have been shown to be efficacious in reducing the incidence and risk of AD and in delaying disease progression. Combs et al. demonstrated that NSAIDs, thiazolidinediones, and PGJ<sub>2</sub>, all of which are PPAR $\gamma$  agonists, inhibited the  $\beta$ -amyloid-stimulated secretion of inflammatory products by microglia and monocytes. PPAR $\gamma$  agonists were shown to inhibit the  $\beta$ -amyloid-stimulated expression of the genes for IL-6 and TNF- $\alpha$  and the expression of COX-2. Heneka et al. demonstrated that microinjection of LPS and IFN- $\alpha$  into rat cerebellum induced iNOS expression in cerebellar granule cells and subsequent cell death. Coinjection of PPAR $\gamma$  agonists (including troglitazone and 15d-PGJ<sub>2</sub>) reduced iNOS expression and cell death, whereas coinjection of a selective COX inhibitor had no effect. Overall, work in AD seems to suggest that PPAR $\gamma$  agonists can modulate inflammatory responses in the brain and that NSAIDs may be helpful in AD as a result of their effect on PPAR $\gamma$ .

From the previously exposed herein, it can be concluded:

Till now, there are no antecedents of PPAR- $\gamma$  agonist compounds isolated from medicinal plants. Nowadays, there are no drugs, compositions or medicines with these properties for the treatment of autoimmune diseases.

Whereas, this new composition is able to reduce the pro-inflammatory cytokines production, that are increased in autoimmune and neurodegenerative diseases.

Additionally, the composition of the present invention has low toxicity, and does not exhibit any harmful side effects.

Given the current "State of Art" in Science, the use of said composition cannot be deduced by an expert in the field, wherein said composition is directed for the above mentioned diseases, with said properties, maintaining the immune tolerance, without causing adverse effects, as occurs with other substances that are currently used for these diseases.

*Andrographis paniculata* (Nees), is a medicinal plant pertaining to the Acanthaceae family native to Asia, India, Malaysia, China, Korea and elsewhere. In these countries it has been widely employed for their beneficial effects of the fresh and dried plant or its components in different diseases, such as common cold, liver conditions, diabetes, etc.

## BRIEF DESCRIPTION OF THE FIGURES

Figure 1. Is a representative graph showing the PAF induced calcium displacement measured through the ratio 340/380 nm, using HL-60 cells marked with FURA2-AM indicator. The cells were differentiated with retinoic acid alone, or in presence with the composition of the present invention (3.5 µg/mL), as described in examples 1-2, disclosed hereinafter.

Figure 2. Shows a bar diagram depicting the relative luciferase activity in HL-60 cells transfected with a vector that contains the promoter of PPAR $\gamma$  and the effect of the composition of the present invention.

- Figure 3. Is a bar graph showing the inhibition of IL-2 and INF-gamma concentration in T cells activated with concanavalin CONA by the composition of the present invention.
- 5 Figure 4. Shows the inhibition of the  $\text{I}\kappa\text{B}\alpha$  degradation by the composition of the present invention and the bar diagram shows the inhibition percentage of the composition of the present invention on the relative luciferase activity in HL-60 cells transfected with a vector that contains the  $\text{NF}\kappa\text{B}$  promotor.
- 10 Figure 5. Is a microphotography showing the *in vitro* inhibition of  $\beta$  amiloid formation by the composition of the present invention, using the thioflavin staining.

## DETAILED DESCRIPTION OF THE INVENTION

15

In the present application, a new composition is described, that induces PPAR-gamma agonistic effects and inhibits the activation of the transcription factor  $\text{NF-kappaB}$  using a mixture of andrographolides extracted from *Andrographis paniculata* by applying the procedure disclosed herein.

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## DESCRIPTION OF THE ANDROGRAPHOLIDES COMPOSITION

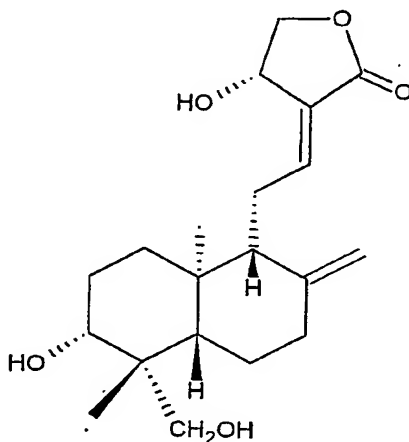
The composition claimed in the present application, comprises a mixture of diterpenic labdanes, obtained from an extract of *Andrographis paniculata* dried  
25 extract, having the following general formulae:

$\text{C}_{20}\text{H}_{30}\text{O}_5$	Andrographolide
$\text{C}_{20}\text{H}_{30}\text{O}_4$	14-Deoxiandrographolide
$\text{C}_{26}\text{H}_{41}\text{O}_8$	Neoandrographolide

The chemical structure and characterization of these andrographolide compounds is as follows:

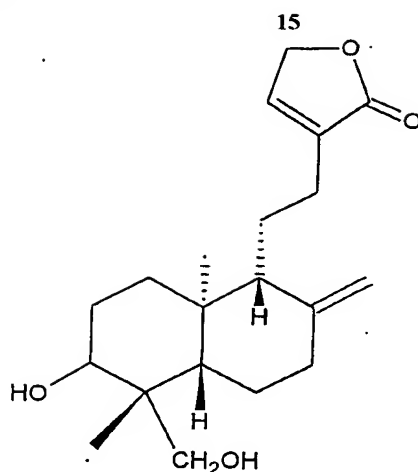
- Andrographolide

- 5                    i.      General formula:  $C_{20}H_{30}O_5$
- ii.      Molecular weight: 350.46
- iii.     Molecular nomenclature: 3-[2-[decahydro-6-hydroxy-5-(hydroxy-methyl)-5,8a-dimethyl-2-methylen-1-naphthalenyl]ethylidene]di-hydro-4-hydroxy-2(3h)-furanone.
- 10                   iv.      Molecular structure:



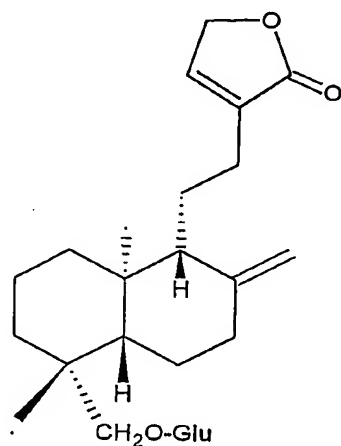
- 14-Deoxyandrographolide

- i.      General formula:  $C_{20}H_{30}O_4$
- 15                   ii.      Molecular weight: 336.46
- iii.     Molecular structure:



- Neoandrographolide

- 5            i.     General formula:  $C_{20}H_{41}O_8$   
              ii.     Molecular weight: 326.46  
              iii.    Molecular structure:



10 A representative extract of the present composition, is constituted by a mixture of the following Andrographolides: Andrographolide, 14-Deoxyandrographolide, and Neoandrographolide. Wherein said individual components are contained approximately 20 to 40% w/w of Andrographolide, about 3 to 6% w/w of 14-Deoxyandrographolide, and about 0.2 to 0.8% w/w of Neoandrographolide in the

dried extract. Preferably these compounds are contained from about 25 to 35% w/w of Andrographolide, from about 4.5 to 5.5% w/w of 14-deoxyandrographolide, and approximately 0.4 to 0.8% w/w of Neoandrographolide in the final extract.

5

In a more preferred embodiment, the novel extract comprises:

	Andrographolide	24.6%
	14-Deoxyandrographolide	4.8%
10	Neoandrographolide	0.6%

Said formulation is acceptable for manufacturing medicines which can be administered with a pharmaceutically acceptable carrier, i.e. a tablet form, administered in a dose comprising approximately 1 to 6.5 mg/kg BW/day of the andrographolides mixture.

15

- a) 1 - 5 mg Andrographolide/kg per day
- b) 0.2 - 1 mg 14-Deoxyandrographolide/kg per day
- c) 0.02 - 0.12 mg NeoAndrographolide/ kg per day.

20

Without affecting other formulation and administration embodiments, those herein disclosed, contribute efficiently and effectively for the treatment of autoimmune diseases already mentioned and also for treating Alzheimer's disease, according to the examples disclosed herein.

25

Therefore, both the composition and its pharmaceutical formulation particularly when administered in the tablet form and in the above indicated doses, provide a medicine for treating a variety of autoimmune diseases such as: inflammatory

disorders; particularly, diabetes mellitus, inflammatory bowel disease; autoimmune diseases (lupus erithematosus, multiple sclerosis and rheumatoid arthritis).

Due to its mechanism of action, the compounds and the pharmaceutical composition herein disclosed can also be useful for treating AIDS and tissue and organ rejection.

The pharmaceutical composition that can be manufactured with the composition of the present invention, specially according to the revealed formulation, can correspond to enteral, parenteral, dermic, ocular, nasal, otic, rectal, vaginal, urethral, bucal, pharyngeal-tracheal-bronchial pharmaceutical forms.

#### METHOD OF OBTAINING AND ANALYSIS OF *ANDROGRAPHIS PANICULATA* RAW MATERIAL

Active ingredient:	<i>Andrographis paniculata</i> Nees (Burm. f.)
Family:	Acantaceae
Used part:	herba

The green leaves, stems and higher parts, organically cultivated under supervision of the inventor including the seeds are sun dried. All foreign materials are manually removed and the raw material is cut into 1-1.5 cm size pieces, which are stored in a ventilated area. Routine analysis is carried out in order to asses the identity: macro and microscopic analysis, organoleptic parameters and TLC analysis (thin layer chromatography) is performed according to European Pharmacopoeia.

METHOD FOR OBTAINING THE *ANDROGRAPHIS PANICULATA* DRIED EXTRACT

5 The extraction of *A. paniculata* is performed by continuous percolation of the grinded dried plant (aerial part) using a polar solvent in part 1.

10 The duly analyzed drug material is grinded to suitable particle size in a knife-hammer mill (0.8 cm<sup>2</sup>). The grinded material is charged into stainless steel percolators and the extraction solution is added at a temperature of 50°C. The percolation time is of approximately 6 days (6x24hours) in two extraction cycles. The percolate is collected in stainless steel tanks until the percolation is completed. The percolate is transferred directly to an evaporation unit in order to eliminate the solvent and most of the water. Evaporation is performed in a LUWA thin-film  
15 evaporator at 140–158°F (60–70°C) and 0.65–0.85 bar vacuum. The evaporation process is performed in 3–4 cycles, where the extract is kept under mixing, 4 times during 30 minutes per day. When the spissum extract has the right content of water, the following analysis are made: ash content, HCL-ash, loss on drying, pH value, TLC identity and HPLC (high performance liquid chromatography), analysis for Andrographolide, 14-Deoxyandrographolide and Neoandrographolide.  
20 Then the spissum extract is transferred to the drying unit. Before drying, The final dried extract is packaged in plastic bags in fiber drums for subsequent analysis.

Method of preparation of *Andrographis Paniculata* 30% extract:

25 Cut and sieved leaves/stem of *Andrographis paniculata* are collected from farms under direct control of the inventors. The aerial parts are analyzed for identity as previously described and then taken for extraction.

The aerial parts are extracted in a Stainless steel extraction unit under vacuum with a low polarity solvent (A), such as N-hexane or chloroform. Following successive

extractions, the solvent is removed and the marc is treated with a second solvent having higher polarity (B) such as Pet ether 40:60 or ethyl acetate, after a single extraction, the solvent is removed and the marc is treated with a solvent (C) having greater polarity such as ethanol or water.

5

The third solvent is recovered and evaporated leaving behind a mass with 30-40 % moisture, the marc is treated with a solvent having low polarity as described previously, the mass is then filtered and dried under vacuum till there is less than 5% moisture. The granules are ground to a fine powder having not less than 30% labdane diterpenes calculated as andrographolides.

10

Details of extraction:

Step 1: Finely cut leaves/stem of *Andrographis paniculata* are loaded in an S S reactor with between 3-5 times w/w of solvent A.

15

Step 2: The herb is extracted for between 4-6 hours repeatedly and the solvent removed.

Step 3: The marc is then treated with a solvent B having higher polarity than solvent A and extracted once for 3-5 hours.

20

Step 4: The solvent B is removed and the marc is then extracted with solvent C having higher polarity than solvents A or B.

25

Step 5: The solvent C is circulated through the marc for 3-5 hours and removed under vacuum to an S S evaporator and the marc is again extracted with solvent C, this process is repeated 3-4 times.

Step 6: The solvent C is recovered from all the washings and the resulting mass pooled together

Step 7: The mass obtained from Steps 4-7 is then treated with Solvents A or B and the residue is dried under vacuum at a temperature not exceeding 60 C.

Step 8: The dried mass that is obtained from Step 7 is powdered using a GMP Grinder having stainless steel meshing between 100-200 ASTM .

Step 9: The powder obtained from Step 8 is sieved through an auto siever and directly filled into sterilized PP bags ready to be sealed.

The powder obtained as described above is analyzed as per the protocols described in this document having 25 to 35% w/w of Andrographolide, from about 4.5 to 5.5% w/w of 14-deoxyandrographolide, and approximately 0.4 to 0.8% w/w of Neoandrographolide in the final extract.

#### IDENTITY of ANDROGRAPHIS PANICULATA – TLC

Test solution: To 1 g herbal extract, 20 ml of methanol is added, shaken for about 1 hour and the methanol is decanted through a filter. The residue is shaken with 20 ml methanol, filtered and mixed with the first extract (making 40ml of test solution).

Reference solutions: 1 Andrographolide (A), 14-Deoxiandrographolide (DA) and Neoandrographolide (NA), dissolved in methanol. 2. Reference-extract treated in the same way as the test-extract. 20-30 ul test solution is applied to a TLC-plate (silica gel GF254 as coating substance) and developed over a path of 15 cm using a mixture of 77 volumes of ethyl acetate, 15 volumes of methanol and 8 volumes of

water (77:15:8). Subsequently, the plate is allowed to dry in air and examined under UV (254nm). The few dark spots of the chromatogram correspond to Andrographolide at a  $R_f$ : 0.65-0.7; 14-Deoxyandrographolide  $R_f$ : 0.75-0.8 and Neoandrographolide,  $R_f$ : 0.60-0.65.

5

#### HPLC METHOD FOR THE QUANTIFICATION OF DITERPENIC LABDANES

10 The three compounds are extracted with acetone (4:1) and then analyzed by HPLC using a reverse phase RP-C18 lirospher column (4x125mm). The mobile phase consists of acetonitrile 26% and phosphoric acid 0.5%, at a rate of 1.1 ml/min, and is detected at 228 nm according to Burgos et al.; 1999, Acta Hort. (ISHS) 501:83-86.

15 The *Andrographis paniculata* dried extract is standardized to a minimum of 30% of total Andrographolides, which comprises approximately 20 to 40% w/w of andrographolide, 3 to 6% w/w of 14-deoxyandrographolide, and 0.2 to 0.8% w/w of neoandrographolide.

20 The composition according to the present invention has not been previously disclosed in the current "state of the art" in science and there are no antecedents about the use of the same in order to solve the described methodological problems concerning autoimmune diseases and AD.

25 The pharmaceutical compositions of this invention may be administered orally or parenterally, and the parenteral administration comprises intravenous injection, subcutaneous injection, intramuscular injection and intraarticular injection.

The correct dosage of the pharmaceutical compositions of the invention will vary depending on the particular formulation, the mode of application, age, body weight and gender of the patient, diet, disease status of the patient, complementary drugs and adverse reactions. It is understood that the ordinary skilled physician will readily be able to determine and prescribe a correct dosage of this pharmaceutical compositions. Preferably, the daily dosage of this pharmaceutical compositions ranges from 1- 6.5 mg of the andrographolides mixture per kg of body weight.

According to the conventional techniques known to those skilled in the art, the pharmaceutical compositions of the present invention can be formulated with a pharmaceutical acceptable carrier and/or vehicle as described above, such as a unit dosage form. Non-limiting examples of the formulations include, but are not limited to, a sterile solution, a solution, a suspension or an emulsion, an extract, an elixir, a powder, a granule, a tablet, a capsule, a liniment, a lotion and an ointment.

The present invention also embraces the pharmaceutical compositions containing Andrographolide, 14-Deoxyandrographolide and Neoandrographolide Labdanes compounds in combination with pharmaceutically acceptable carriers normally employed in preparing such compositions.

In the pharmaceutical compositions of this invention, the pharmaceutically acceptable carrier may be any conventional one described for pharmaceutical formulations, such as lactose, dextrose, sucrose, sorbitol, mannitol, starch, gum acacia, calcium phosphate, alginate, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, water, syrup, methyl cellulose, Hydroxypropylmethylcellulose (HPMC), methylhydroxy benzoate, propylhydroxy benzoate, talc, stearic acid, magnesium and mineral oil, but not limited thereto. Additionally, the pharmaceutical compositions of the present invention may contain any of a wetting agent, sweetening agent, emulsifying agent, suspending

agent, preservatives, flavors, perfumes, lubricating agent, or mixtures of these substances.

Typically, the pharmaceutical compositions contains from 20-40 %, preferably  
5 from 25 to 35%, and most preferably 30% w/w of Andrographolide ,  
14-Deoxyandrographolide and Neoandrographolide Labdanes of the mixture, and  
the pharmaceutically acceptable carriers.

The pharmaceutical composition of the present invention can be administered to  
10 mammals in need thereof, via oral route administered singly or as a divided dose.  
Thus, for oral administration, the compounds can be combined with a suitable solid  
carrier to form capsules, tablets, powders. Additionally, the pharmaceutical  
compositions may contain other components such as flavourants, sweeteners,  
excipients and the like.

15 Additionally, the present invention provides a method for treating patients with the  
composition containing the andrographolides mixtures which comprises:  
intravenous administering of the solution and orally administering the tablets  
comprising the composition of the present invention to patients in need thereof.  
20 The preferred dosage of the injection solution formulation is about 60 to 210  
mg/day, most preferably, 60-80 mg/day, of the composition per day in one, two or  
three injections.

The present formulation in the injectable solution form comprises 8-16 mg  
25 approximately of the composition per ml. When administered to patients, the  
composition is preferably diluted to about 1:5 to 1:10 volume of 0.9% saline  
solution.

The following examples are illustrative, but do not limit the scope of the present invention. Reasonable variations, such as those occurring to a reasonable artisan, can be made herein without departing from the scope of the present invention.

5 The pharmaceutical composition of the present invention is suitable for preparation in a scale typical for pharmaceutical industry as well as for smaller measure. Following conventional techniques of the pharmaceutical industry involving wet granulation, dry granulation, direct compression, fluid bed granulation, when necessary, for tablet forms, as appropriate, to give the desired oral, or parenteral  
10 products.

The percentages indicated in the following examples are all given by weight.

#### EXAMPLES

15 Exemplary of a typical method for preparing a tablet containing the active agents is to first mix the active agent with a binder such as gelatin, ethyl cellulose, or the like. Wherein the mixing is suitably carried out in a standard V-blender and usually under anhydrous conditions. Next, the just prepared mixture can be slugged  
20 through conventional tablet machines and the slugs fabricated into tablets. The freshly prepared tablets are coated, with suitable coatings including shellac, methylcellulose, carnauba wax, styrene-maleic acid copolymers, and the like.

For oral administration, the compressed tablets containing from 30 mg up to 40 mg  
25 of the andrographolide mixture are manufactured according to the above disclosed methods of manufacturing techniques well known to the art and set forth in Remington's Pharmaceutical Science, Chapter 39, Mack Publishing Co., 1965.

The preferred pharmaceutical compositions of the present invention formulations are shown in some of the following Examples.

#### EXAMPLE 1

- 5 Pharmaceutical composition for preparing a tablet of the present invention, using the andrographolide mixture contained in the dried extract obtained from the herb *Andrographis paniculata* Nees.

10	Ingredients: Per tablet	mg.
	Dried Extract (Andrographolides mixture)	135.0
	Potato starch	168.8
	Talc	106.9
	Gelatin	11.5
15	Magnesium stearate	5.6
	Hydroxypropyl methyl cellulose	3.5
	Silicon dioxide, anhydrous	2.0
	Polyethylene glycol	0.7
20	Carbonate, calcium (qsp.)	16

To formulate the tablet uniformly blend the dried extract (Andrographolides mixture) active compound, potato starch, talc, gelatin, hydroxypropyl methyl cellulose, silicon dioxide, anhydrous, polyethylene glycol, and calcium carbonate under dry conditions in a conventional V-blender until all the ingredients are uniformly mixed. The mixture is then passed through a standard light mesh screen, dried in an anhydrous atmosphere and then blended with magnesium stearate, and compressed into tablets, and coated with shellac. Other tablets containing from 116 to 162 mg, are prepared in a similar fashion.

## EXAMPLE 2

Pharmaceutical composition for preparing a capsule of the present invention, using the dried extract obtained from the herb *Andrographis paniculata* Nees.

5		
	Ingredients: Per capsule	mg.
	Dry Extract, (Andrographolides mixture)	135.0
	Potato starch	168.8
10	Talc	106.9
	Gelatin	11.5
	Magnesium stearate	5.6
	Hydroxypropyl methyl cellulose	3.5
	Silicon dioxide, anhydrous	2.0
15	Polyethylene Glycol	0.7
	Carbonate, calcium	16

The manufacture of capsules containing from 30 mg to 40 mg of andrographolide mixture for oral use consists essentially of mixing the Dried Extract, (Andrographolides mixture) with a carrier and enclosing the mixture in a polymeric sheath, usually gelatin or the like. The capsules can be in the art known soft form of a capsule made by enclosing the compound in intimate dispersion within an edible, compatible carrier, or the capsule can be a hard capsule consisting essentially of the novel composition mixed with a nontoxic solid such as talc, calcium stearate, calcium carbonate, or the like. Exemplary of a typical use for employing a capsule containing 30mg of 40mg for use as therapeutically indicated.

- The dose administered, whether a single dose, multiple dose, or a daily dose, will of course, vary with the particular compound of the invention employed because of the varying potency of the compound, the chosen route of administration, the size of the patient and the nature of the disease condition. The administered dose
- 5 corresponds to a general oral dose of 80 to 160 mg daily, with the oral dose of normally 120 mg three times per day; the usual intravenous dose of 60 to 80 mg, followed if indicated by 70 to 100 mg at a later period, and the usual intramuscular dose of 70 to 100 mg every 24 hours, with 1 to 2 injections per day.
- 10 The novel and useful pharmaceutical compositions comprising the Dried Extract, (containing a Andrographolides mixture) of the invention are adaptable for the administration for their physiological expected effects from drug delivery systems, such as skin delivery systems, gastrointestinal drug delivery devices, and the like, wherein the delivery device is manufactured from naturally occurring and synthetic
- 15 polymeric materials. Representative of materials acceptable for the fabrication of drug delivery systems containing the compounds for controlled drug administration include materials such as polyvinyl chloride, polyisoprene, polybutadiene, polyethylene, ethylene-vinyl acetate copolymers, polydimethylsiloxane, hydrophilic hydrogels of esters of acrylic and methacrylic acid, polyvinyl acetates,
- 20 propylene vinyl acetate copolymers, and the like.

### EXAMPLE N°3

- Shellac covered tablets containing the above indicated composition are prepared following conventional techniques of the pharmaceutical industry involving
- 25 mixing, granulating, and compressing, when necessary, for tablet forms.

Specifically the composition of example 1 is thoroughly mixed with a sufficient amount of *Andrographis paniculata* dried extract. For the manufacture of tablets comprising Andrographolide, 14-Deoxyandrographolide and Neoandrographolide,

the mixture is compressed in a direct form with the inactive ingredients mentioned in example N°1, and subsequently covered with shellac, accordingly.

#### EXAMPLE N°4

5 HL-60 cell differentiation induced by the composition.

Chemicals: May Grunwald-Giemsa, NBT, retinoic acid, cytochalasin B, penicillin, streptomycin, glutamine, fetal bovine serum (Sigma). RPMI 1640 (GIBCO), Fetal bovine serum from Boehringer Mannheim, all trans-retinoic acid and  
10 andrographolide was from Aldrich. The other isolates were supplied from Amsar Pvt. Ltd., India. FURA2-AM was purchased from Molecular Probes (USA). Nitroblue tetrazolium was from Sigma.

Cell Culture: *HL* 60 cells were grown in RPMI 1640 medium supplemented with  
15 20% heat-inactivated fetal bovine serum, 2 mM glutamine, 100 IU/ml penicillin, and 100 pg/ml streptomycin at 37 °C in a humidified atmosphere containing 5% CO<sub>2</sub>. Cells were seeded twice weekly at  $3 \times 10^5$  cells/ml. Differentiation was induced by adding 100 nM of all-trans-retinoic acid, alone or combined with the composition (17.5 µg/ml) and assessed by the change in morphology after May  
20 Grunwald-Giemsa staining and the ability to reduce NBT' (11). Undifferentiated cultures contained less than 3% NBT positive cells. Differentiated cultures were studied after 5 days of retinoic acid treatment.

#### Calcium Measurement [Ca<sup>2+</sup>]<sub>c</sub>

25 HL-60 granulocytes ( $2 \times 10^7$ /ml) were loaded with 2 µM fura-2/AM in Ca<sup>2+</sup> medium plus 0.1% bovine serum albumin for 45 min at 37 °C, then diluted to 10<sup>7</sup> cells/ml and kept on ice. Just before use, 0.5 ml of this cell suspension was centrifuged and re-suspended in 2.4 ml of the indicated medium including 5 µg/ml cytochalasin B. Fura-2 fluorescence (*F*) was measured in a thermostated cuvette

(37°C) (LS55 fluorimeter, Perkin-Elmer Corp.) at 340 and 380 nm excitation and 505 nm emission wavelength.

#### EXAMPLE N° 5

##### 5 Inhibition of IL-2 and IFN- $\gamma$ production in T cells by the composition.

Chemicals: concanavaline A and RPMI 1640 medium from Sigma.

10 Cell cultures: Rockefeller mice were sacrificed by ether and the popliteal ganglia and spleen were placed in a Petri plaque containing 5 ml of a medium culture RPMI 1640. The lymph cells were obtained by disrupting these organs in a RPMI 1640 sterile solution, and the lymphocytes were re-suspended in 1 ml of RPMI 1640 medium and quantified with a Neubauer chamber. Finally, the suspension of lymphocytes was adjusted to a concentration of  $4 \times 10^6$  cells per ml RPMI. Once  
15 obtained, the lymphocytes were cultivated in presence or absence of the composition. For this purpose, culture plaques of poliestyrene of 24 well (2 ml each); containing 1 ml cells and different concentrations of the composition and 1 ml of the mitogen concanavaline A (CONA, 0  $\mu$ g/ml and 10  $\mu$ g/ml) were used.

20 The plaques were incubated in a oven at 37°C in an atmosphere of humidity 5% and CO<sub>2</sub> for 24h, then a sample of 2 ml each were added and centrifuged for one minute at 3200 rpm. Afterwards, the cells were freezed in 0.6 ml aliquots and the cytokines detected with ELISA (Enzyme Linked Immuno Sorbent Assay).

##### 25 ELISA for IL-2 and IFN- $\gamma$

Chemicals: IL-2 & IFN gamma from Pharmingen; TMB from Pierce, H<sub>2</sub>O<sub>2</sub> and H<sub>2</sub>SO<sub>4</sub> from Merck.

For the determination of cytokines (IL-2 and IFN) a first antibody that captures the anti-antigen; a second antibody that conjugates to a peroxidase enzyme and a standard solution for a calibration curve was used. ELISA "high binding" 96 wells polystyrene plates were used. 100 µl per well of the first antibody was diluted in carbonate buffer pH 9.5 with the aim to facilitate the sticking to the well, and incubated overnight at 4°C to assure the binding to the solid part. Afterwards, the content of the well was eliminated and washed with 300 µl per well with Tween 20; 0, 05 % p/v and PBS pH 7.0 three times. Then, the well was blocked with 200 µl of fat free milk 5% and PBS, and incubated for 1 hour at room temperature.

After completion, the content well was eliminated and washed as explained already. Then, the test samples where added containing, the antigen, 100 µl per well in duplicate, and 100 µl of a calibration curve specific for the cytokine and incubated for 2 hours at room temperature. Afterwards, the content well was eliminated and washed 5 times according to the latter protocol. Then, the second conjugated antibody was added with the peroxidase enzyme and diluted in a PBS and SBF solution 10%, and 100 µl was plated per well and left at room temperature during one hour. Then, washed 7 times and revealed with a TMB solution and H<sub>2</sub>O<sub>2</sub>, 100 µl per well and developed after 30 minutes in darkness, the reaction was stopped with H<sub>2</sub>SO<sub>4</sub> 2M; 50 µl per well. The result of the reaction was measured by ELISA with a 450 nm filter (Elx800 universal Microplate Reader, BioteK).

#### EXAMPLE N°6

##### Stimulation of PPAR-γ receptor by the COMPOSITION

Chemicals: Dimethylsulfoxide purchased from Merck. All other reagents were purchased from PROMEGA.

Transcription Assays: HL-60 cells were transfected with pCMX-hPPAR<sub>γ</sub>1, the human PPAR<sub>γ</sub>1 expression vector under control of a cytomegalovirus promoter.

Luciferase and  $\beta$ -galactosidase activities were determined; and the luciferase activity was normalized to the  $\beta$ -galactosidase standard in HL-60.

Plasmids: The plasmid expressing the GAL4-DNA binding domain (DBD) and the mPPAR $\gamma$  ligand binding domain (pGAL4DBD-mPPAR $\gamma$ ) was constructed by inserting the mouse PPAR $\gamma$ 1 ligand binding domain (from amino acids 162-475), isolated as a ScaI/BamHI fragment from pGBTmPPAR $\gamma$ 1 in-frame into pCMXGal4 DBD.

- 10 The cells were treated with DMSO or 17.5  $\mu$ g/ml composition of the present invention, and the luciferase activity was measured by chemiluminescence.

#### EXAMPLE N°7

NF- $\kappa$ B inhibition in neutrophils by the composition

15 Chemicals: dimethylsulfoxide (DMSO) from Merck; bovine fetal serum and RPMI-1640 medium from Gibco, USA; nitro tetrazolium blue from Sigma, pRL-TK, pGL3 and Dual-Luciferase Reporter Assay System (Promega); Fugene6 from Roche;

#### 20 Cell culture

- A cellular myeloid HL-60 line from acute myeloid leukemia was used. This cells can differentiate in the presence of dimethylsulfoxide 1.3% (DMSO) (Santos-Beneit *et al.*, 2000). The cells are kept in RPMI-1640 medium supplemented with 2 mM L-glutamine, 10% of bovine fetal serum inactivated by temperature and antibiotics with 5% CO<sub>2</sub> at 37°C. The cells are differentiated to neutrophils by incubating the neutrophils with 1.3% of DMSO for 4 days. The differentiated cells are analyzed with nitro tetrazolium blue (NBT).

## Transfection of the NFkB-pGL3 vector in HL-60 cells and luciferase measurement

HL-60 cells were cultivated and differentiated to neutrophils for 4 days, as described elsewhere. At day 4, the cells were transfected with the pGL3-NFkB vectors and as an internal control of the transfection a pRL-TK (Promega) vector was used, which is an expression vector containing a thymidine kinase promoter of herpes simplex virus, which allows the expression of moderate levels of *Renilla* luciferase. These vectors are transfected to cells by a system based on liposomes (Fugene6, Roche). Once the transfection is done, the cells are kept for 24 h, and then stimulated by PAF or fMLP at different times, in presence or absence of the composition of the present invention. Then, cellular extracts are kept at  $-70^{\circ}\text{C}$  till the measurement of the activity of luciferase. The activity of luciferase is measured by chemiluminescence, with the commercial system Dual-Luciferase Reporter Assay System (Promega) that possesses the substrates of the enzymes firefly (pGL3) and *Renilla* (pRL) luciferase.

### I $\kappa$ Ba Immunoblot

Chemicals: fMLP; PMSF and PAF were purchased from Calbiochem. Tris, NaCl, NP-40, deoxycholate, sodium dodecylsulphate, iprotease inhibitors, mercaptoethanol from Merck.

The neutrophils were preincubated for 10 minutes, and then stimulated with fMLP (0.1  $\mu\text{M}$ ) and PAF (0.1  $\mu\text{M}$ ) for 60 min. For the analysis of proteins, the cells were lysed in a lysis tampon (50 mM Tris, pH 8.0, 150 mM NaCl, 1% NP-40, 0.5% deoxycholate, 0.1% sodium dodecylsulphate, 1mM  $\text{Na}_2\text{VO}_4$ , 1mM PMSF and 10  $\mu\text{g/ml}$  de iprotease inhibitors). The proteins were quantified by the Bradford method, resolved by electrophoresis in polyacrylamide gel in denaturized conditions (SDS/PAGE) 12%, and electro transferred to a nitrocellulose

membrane. The membrane was incubated with anti-IkB $\alpha$  antibodies, followed by a secondary peroxidase conjugated antibody and finally visualized with chemiluminescence (ECL). As a control of the quantity of proteins in the gel, the antibodies were treated with *stripping* solution (100 mM 2-mercaptoethanol, 2 % SDS, 62.5 mM Tris-HCl, pH 6.7) and incubated with an anti-actin antibody. Finally, a densitometric analysis with the obtained signals for each antibody was performed.

#### EXAMPLE N°8

##### 10 Inhibition of $\beta$ -amyloid formation by the composition in wild type rats

##### Amyloid formation

To check the amyloid formation two complementary techniques, thioflavine-T fluorescence (Levine, 1993; Soto et al., 1995; Reyes *et al.*, 1997, Inestrosa *et al.*, 2000) and Congo red binding (Alvarez *et al.*, 1998) were used. Briefly, the assay of thioflavin-T is based on fluorescence emission of thioflavin when it binds to amyloid fibrils, showing an increasing of emission at 482 nm when is excited at 450 nm. The Congo red assay is a very specific quantification assay to determine the amount of amyloid formed. These techniques are currently used to verify the specific amyloid formation.

20

#### EXAMPLE 9

##### Description of a compound from the composition of Andrographolides

A representative composition of the present invention is a pharmaceutical formulation in tablets, which supplies the following mixture of compounds:

Andrographolide	24.6%,
14-Deoxyandrographolide	4.8%

Neoandrographolide 0.6%

for the subsequent manufacture of the different pharmaceutical forms, and applied in the following doses:

5

- a) 1-5 mg andrographolide/kg per day
- b) 0.2 – 1 mg 14-Deoxyandrographolide/kg per day
- c) 0.02-0.12 mg neoandrographolide/kg per day.

## 10 EXAMPLE N°10

Clinical efficacy of an oral formulation for the treatment of lupus erythematosus

Using the mixture of andrographolides described in example 7, a normalization of the symptoms due to lupus occurs following 3 months of administration. In  
15 addition, the composition does not interfere with the normal rebuilding effects of other traditional non-steroidal anti-inflammatory agents.

## EXAMPLE N°11

Clinical efficacy of an oral formulation for the treatment of multiple sclerosis

20

Using the mixture of andrographolides described in example 7, normalization in the symptoms of the disease occurs following 3 months of treatment of the composition of the present invention. In addition, the composition does not interfere with other treatments.

25

## EXAMPLE N°12

Clinical efficacy of an oral formulation for the treatment of arthrosis and rheumatoid arthritis

Using the mixture of andrographolides described in example 7, normalization of joint stiffness due to osteoarthritis occurs following 3 months, in the presence or absence of glucosamine or chondroitin sulphate or other anti-inflammatory drugs. In addition, the composition does not interfere with the normal joint rebuilding effects of these two proteoglycan constituents, unlike traditional non-steroidal anti-inflammatory

#### EXAMPLE N°13

Clinical efficacy of an oral formulation for the treatment of Diabetes mellitus

Using the mixture of andrographolides described in example 7, normalization in the sugar levels occurs following five weeks. In addition, the composition does not interfere with the normal rebuilding effects of other sugar reducing agents.

#### EXAMPLE N°14

Clinical efficacy of an oral formulation for treating AIDS

An oral formulation as described in Example 7 is administered to patients who are HIV positive. Normal CD4 counting is restored within 3 months of treatment.

#### EXAMPLE N°15

Clinical efficacy of an oral formulation for treating Alzheimer's disease

An oral formulation as described in Example 7 is administered to patients who have manifested an early stage of Alzheimer's Disease (AD), as diagnosed by their practitioner and confirmed by an independent board-certified neurologist. Two weeks before the clinical trial, the patients undergo appropriate psychoneurological tests such as the Mini Mental Status Exam (MMSE), the Alzheimer Disease

Assessment Scale (ADAS), the Boston Naming Test (BNT), and the Token Test (TT).

Neuropsychological tests are repeated on Day 0, 6 weeks and 3 months of the clinical trial. The tests are performed by neuropsychologists who are not aware of the patient's treatment regimen.

Patients are randomly assigned to the test formulation or placebo at the start of the study. The test formulation and placebo are taken orally one or two times per day. Treatment for conditions such as diabetes, hypertension, etc. is allowed during the study. Scores are statistically compared between the test formulation and the placebo for each of the three observational periods. Without treatment, the natural course of AD is a significant deterioration in the test scores during the course of the clinical trial. Patients treated with the composition as described in the formulation are considered improved if the patients' scores remain the same or improve during the course of the clinical trial.

The patients shall receive randomly the composition or a placebo at the beginning of the study. The composition and placebo are administered twice a day. During the study the patients are allowed to be treated for conditions as diabetes, hypertension, etc.. The composition and placebo results are statistically compared for all the study periods. Patients using placebo show a significant cognitive deterioration. The patients treated with the composition ameliorate in a considerable way the test scores.

From the foregoing description, it is obvious that one of ordinary skill in the art can not easily ascertain the essential characteristics of the present invention, and without departing from the spirit and scope thereof, can make various changes and/or modifications to the invention for adapting it to various usages and

conditions. As such, these changes and modifications are properly, equitably and intended to be, within the full range of equivalence of the following claims.

## CLAIMS

- 5 1. Composition, comprising a mixture of diterpenic Labdanes obtained from a plant *Andrographis paniculata* dried extract, whose general formulae are:

$C_{20}H_{30}O_5$  Andrographolide

$C_{20}H_{30}O_4$  14-Deoxiandrographolide

$C_{26}H_{41}O_8$  Neoandrographolide

10

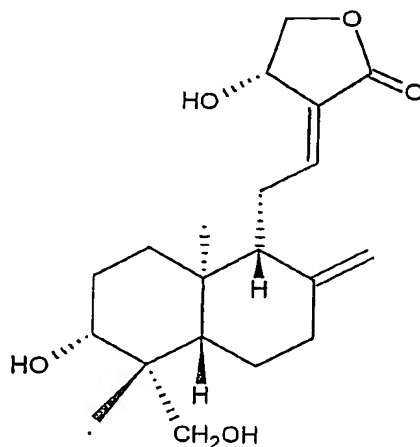
2. The composition, according to claim 1, wherein the andrographolide component is characterized by:

i.) general formula:  $C_{20}H_{30}O_5$

ii.) molecular weight: 350.46

- 15 iii.) molecular nomenclature: 3-[2-[decahydro-6-hydroxy-5-(hydroxymethyl)-5,8-dimethyl-2-methylene-1-naphthalenyl]ethylidene]-dihydro-4-hydroxy-2(3h)-furanone,

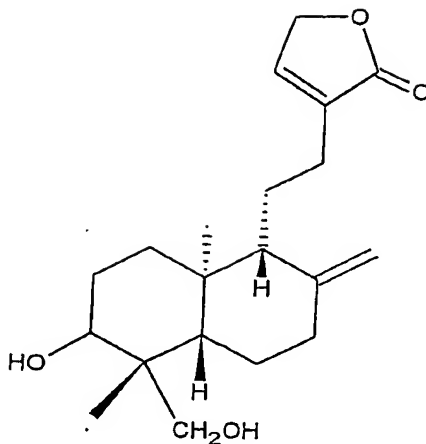
iv.) molecular structure:



20

3. The composition, according to claim 1, wherein the 14-Deoxiandrographolide component is characterized by:

- i.) general formula:  $C_{20}H_{30}O_4$
- ii.) Molecular weight: 336.46
- iii.) Molecular nomenclature:
- iv.) Molecular structure:

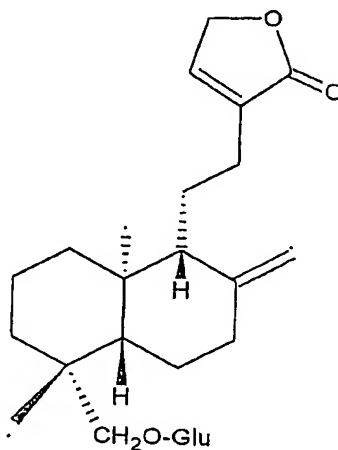


5

4. The composition, according to claim 1, wherein the neoandrographolide component is characterized by:

- i.) general formula:  $C_{26}H_{41}O_8$
- ii.) molecular weight: 345.89
- iii.) molecular nomenclature:
- iv.) molecular structure:

10



5. Use of the composition, according to claim 1, characterized in that it is useful  
for preparing a medicine, drug, pharmaceutical.

6. Use of the composition, according to claim 1, characterized in that it is useful  
5 for preparing a medicine suitable for treating autoimmune diseases.

7. Use of the composition, according to claim 1, characterized in that it is  
particularly useful for preparing a medicine suitable for treating rheumatoid  
arthritis.

10

8. Use of the composition, according to claim 1, characterized in that it is  
particularly useful for preparing a medicine suitable for treating lupus  
exanthematous.

15 9. Use of the composition, according to claim 1, characterized in that it is  
particularly useful for preparing a medicine suitable for treating multiple sclerosis.

10. Use of the composition, according to claim 1, characterized in that it is useful  
for preparing a medicine suitable for preventing and treating Alzheimer's disease.

20

11. Use of the composition, according to claim 1, characterized in that it is  
particularly useful for preparing a medicine suitable for treating asthma and  
allergies.

25 12. Use of the composition, according to claim 1, characterized in that it is  
particularly useful for preparing a medicine suitable for treating psoriasis.

13. Use of the composition, according to claim 1, characterized in that, it is particularly useful for preparing a medicine suitable for treating the systemic dermatomyocytis.

5 14. Use of the composition, according to claim 1, characterized in that, particularly, it is useful for preparing a medicine suitable for treating osteoarthritis.

15. Use of the composition, according to claim 1, characterized in that it is useful for preparing a medicine suitable for treating acquired immune deficiency  
10 syndrome (AIDS).

16. Use of the composition, according to claim 1, characterized in that it is useful for preparing a medicine suitable for treating diabetes mellitus.

15 17. Use of the composition, according to claim 1, characterized in that it is useful for preparing a medicine suitable for treating the rejection in patients with tissue and organ transplants.

18. Pharmaceutical compositions, characterized in that it comprises the  
20 composition according to claim 1 and a pharmaceutically acceptable carrier.

19. The pharmaceutical composition, according to claim 18, characterized in that the diterpenic Labdanes mixture comprises from 20 to 40% w/w of Andrographolide, from about 3 to 6% w/w of 14-Deoxyandrographolide, and from  
25 about 0.2 to 0.8% w/w of Neoandrographolide, in the final dried extract.

20. The pharmaceutical composition, according to claim 19, characterized in that the diterpenic Labdanes mixture comprises from about 25 to 35% w/w of

Andrographolide, from about 4.5 to 5.5% w/w of 14-Deoxyandrographolide, and from about 0.4 to 0.8% w/w of Neoandrographolide in the final dried extract.

21. The pharmaceutical composition, according to claim 20, characterized in that the diterpenic Labdanes mixture comprises 24.6% w/w of Andrographolide, 4.8% w/w of Deoxyandrographolide, and 0.6% w/w of Neoandrographolide, based in the final dried extract.

22. Pharmaceutical composition, according to claims 18-21, characterized in that it corresponds to a pharmaceutical formulation in tablet form.

23. Pharmaceutical composition, according to claim 22, characterized in that it is orally administered contributing with the following doses for the following molecules:

- a) 1-5 mg andrographolide/kg per day
- b) 0.2 – 1 mg 14-deoxyandrographolide/kg per day
- c) 0.02-0.12 mg Neoandrographolide/kg per day.

24. Use of the pharmaceutical composition, according to claims 22 and 23, characterized in that it is useful for treating autoimmune diseases.

25. Use of the pharmaceutical composition, according to claims 22 and 23, characterized in that it is useful for treating rheumatoid arthritis.

26. Use of the pharmaceutical composition, according to claims 22 and 23, characterized in that it is useful for treating lupus exanthematous.

27. Use of the pharmaceutical composition, according to claims 22 and 23, characterized in that it is useful for treating multiple sclerosis.

28. Use of the pharmaceutical composition, according to claims 22 and 23, characterized in that it is useful for preventing and treating Alzheimer's disease.

5 29. Use of the pharmaceutical composition, according to claims 22 and 23, characterized in that it is useful for treating asthma and allergies.

30. Use of the pharmaceutical composition, according to claims 22 and 23, characterized in that it is useful for treating psoriasis.

10

31. Use of the pharmaceutical composition, according to claims 22 and 23, characterized in that it is useful for treating systemic dermatomyocytis.

15 32. Use of the pharmaceutical composition, according to claims 22 and 23, characterized in that it is useful for treating osteoarthritis.

33. Use of the pharmaceutical composition, according to claims 22 and 23, characterized in that it is useful for treating the acquired immune deficiency syndrome (AIDS).

20 34. Use of the pharmaceutical composition, according to claims 22 and 23, characterized in that it is useful for treating diabetes mellitus.

25 35. Use of the pharmaceutical composition, according to claims 22 and 23, characterized in that it is useful for treating rejection of organs and tissues in transplanted patients.

36. Pharmaceutical compositions, according to claim 18, characterized in that they can be in suitable enteral, parenteral, dermic, ocular, nasal, otic, rectal, vaginal, urethral, bucal, pharyngeal-tracheo-bronchial pharmaceutical forms, wherein the

pharmaceutical composition comprises a dried extract containing a diterpenic Labdane mixture of andrographolide, 14-deoxyandrographolide and Neoandrographolide.

5 37. The pharmaceutical compositions, according to claim 36, characterized in that the diterpenic Labdane mixture comprises from 20 to 40% w/w of Andrographolide, from about 3 to 6% w/w of 14-Deoxyandrographolide, and from about 0.2 to 0.8% w/w of Neoandrographolide, based in the final dried extract weight.

10

38. The pharmaceutical compositions, according to claim 37, characterized in that the diterpenic Labdane mixture comprises from about 25 to 35% w/w of Andrographolide, from about 4.5 to 5.5% w/w of 14-Deoxyandrographolide, and from about 0.4 to 0.8% w/w of Neoandrographolide based in the final dried extract weight.

15

39. The pharmaceutical compositions, according to claim 38, characterized in that the diterpenic Labdane mixture comprises 24.6% w/w of Andrographolide, 4.8% w/w of Deoxyandrographolide, and 0.6% w/w of Neoandrographolide, based in the final dried extract weight.

20

40. Pharmaceutical compositions, according to claim 36, characterized in that they are administered by the corresponding routes, in the following doses:

a) 1-5 mg andrographolide/kg per day

25 b) 0.2 – 1 mg of 14-deoxyandrographolide/kg per day

c) 0.02 – 0.12 mg Neoandrographolide/kg per day.

41. Use of the pharmaceutical composition, according to claims 36 to 40, characterized in that it is useful for treating autoimmune diseases.

42. Use of the pharmaceutical composition, according to claims 36 to 40, characterized in that it is useful for treating rheumatoid arthritis.

5 43. Use of the pharmaceutical composition, according to claims 36 to 40, characterized in that it is useful for treating lupus exanthematous.

44. Use of the pharmaceutical composition, according to claims 36 to 40, characterized in that it is useful for treating multiple sclerosis.

10

45. Use of the pharmaceutical composition, according to claims 36 to 40, characterized in that it is useful for preventing and treating Alzheimer's disease.

15 46. Use of the pharmaceutical composition, according to claims 36 to 40, characterized in that it is useful for treating asthma and allergies.

47. Use of the pharmaceutical composition, according to claims 36 to 40, characterized in that it is useful for treating psoriasis.

20 48. Use of the pharmaceutical composition, according to claims 36 to 40, characterized in that it is useful for treating systemic dermatomyocytis.

49. Use of the pharmaceutical composition, according to claims 36 to 40, characterized in that it is useful for treating osteoarthritis.

25

50. Use of the pharmaceutical composition, according to claims 36 to 40, characterized in that it is useful for treating acquired immune deficiency syndrome (AIDS).

51. Use of the pharmaceutical composition, according to claims 36 to 40,  
characterized in that it is useful for treating diabetes mellitus.

52. Use of the pharmaceutical composition, according to claims 36 to 40,  
5 characterized in that it is useful for treating the rejection of organs and tissues in  
transplanted patients.

## AMENDED CLAIMS

[received by the International Bureau on 21 December 2004 (21.12.04)]

## AMENDED CLAIMS UNDER ART. 19

1. Composition comprising a mixture of  
Diterpenic, Labdanes obtained from an plant *Andrographis*  
5 *paniculata* dried extract, whose general formulae are:

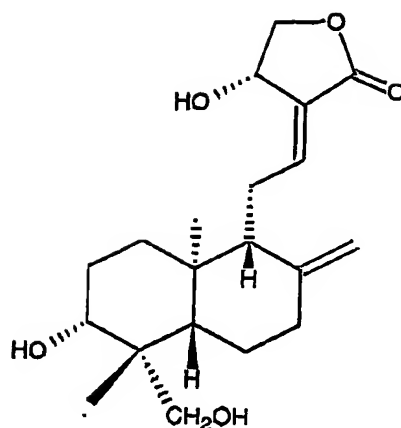
$C_{20}H_{30}O_5$  Andrographolide

$C_{20}H_{30}O_4$  14-Deoxyandrographolide

$C_{26}H_{41}O_8$  Neoandrographolide

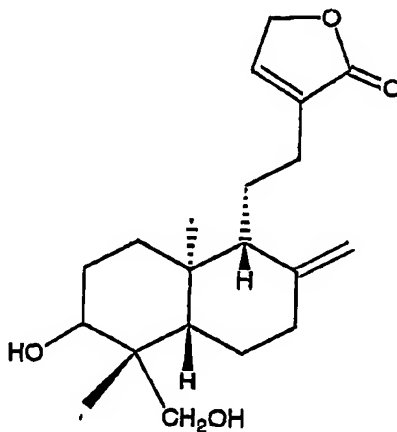
wherein each Diterpenic Labdane is present within the  
10 mixture in an amount from about 20 to about 40% w/w of  
Andrographolide, from about 3 to about 6% w/w of 14-  
Deoxyandrographolide, and from about 0.2 to about 0.8 %  
w/w of Neoandrographolide.

15 2. The composition, according to claim 1,  
wherein the Andrographolide component is characterized by:  
i.) general formula:  $C_{20}H_{30}O_5$   
ii.) molecular weight: 350.46  
iii.) molecular nomenclature: 3-[2-[decahydro-6-hydroxy-5-  
20 (hydroxymethyl)-5,8a-dimethyl-2-methylene-1-naphthalenyl]-  
ethylidene]-dihydro-4-hydroxy-2(3h)-furanone,  
iv.) molecular structure:



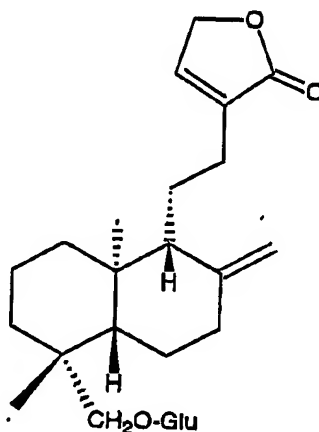
3. The composition, according to claim 1,  
wherein the 14-Deoxiandrographolide component is  
5 characterized by:

- i.) general formula: :  $C_{20}H_{30}O_4$
- ii.) molecular weight: 336.46
- iii.) molecular nomenclature:
- iv.) molecular structure:



4. The composition, according to claim 1, wherein the Neoandrographolide component is characterized by:

- i.) general formula:  $C_{26}H_{41}O_8$
- 5 ii.) molecular weight:
- iii.) molecular nomenclature:
- iv.) molecular structure:



10 5. Use of the composition, according to claim 1, characterized in that it is useful for preparing a medicine, drug, pharmaceutical.

15 6. Use of the composition, according to claim 1, characterized in that it is useful for preparing a medicine suitable for treating autoimmune diseases.

20 7. Use of the composition, according to claim 1, characterized in that it is useful for preparing a medicine suitable for treating rheumatoid arthritis.

8. Use of the composition, according to claim 1, characterized in that it is useful for preparing a medicine suitable for treating lupus exanthematous.

5 9. Use of the composition, according to claim 1, characterized in that it is useful for preparing a medicine suitable for treating multiple sclerosis.

10 10. Use of the composition, according to claim 1, characterized in that it is useful for preparing a medicine suitable for preventing and treating Alzheimer's disease.

15 11. Use of the composition, according to claim 1, characterized in that it is useful for preparing a medicine suitable for treating asthma and allergies.

20 12. Use of the composition, according to claim 1, characterized in that it is useful for preparing a medicine suitable for treating psoriasis.

25 13. Use of the composition, according to claim 1, characterized in that it is useful for preparing a medicine suitable for treating the systemic dermatomyocytis.

14. Use of the composition, according to claim 1, characterized in that it is useful for preparing a medicine suitable for treating osteoarthritis.

5 15. Use of the composition, according to claim 1, characterized in that it is useful for preparing a medicine suitable for treating acquired immune deficiency syndrome (AIDS).

10 16. Use of the composition, according to claim 1, characterized in that it is useful for preparing a medicine suitable for treating diabetes mellitus.

15 17. Use of the composition, according to claim 1, characterized in that it is useful for preparing a medicine suitable for treating the rejection in patients with tissue and organ transplants.

20 18. Pharmaceutical composition, characterized in that it comprises the composition according to claim 1, and a pharmaceutically acceptable carrier.

25 19. The pharmaceutical composition according to claim 18, wherein the diterpenic labdanes are present in an amount from about 25 to 35% w/w of Andrographolide, from about 4.5 to about 5.5% w/w of 14-Deoxyandrographolide, and from about 0.4 to about 0.8 % w/w of Neoandrographolide.

AMENDED SHEET (ARTICLE 19)

20. The pharmaceutical composition according to claim 19, wherein each diterpenic labdanes is present in an amount of 24.6% w/w of Andrographolide, 4.8% w/w of 14-Deoxyandrographolide, and 0.6% w/w of Neoandrographolide.

21. Composition, according to any one of claims 18-20, CHARACTERIZED in that it correspond to a pharmaceutical formulation in tablets form.

22. Pharmaceutical composition, according to claim 21, characterized in that it is orally administered contributing with the following doses for the following molecules:

- a) 1-5 mg andrographolide/kg per day
- b) 0.02-0.5 mg 14-deoxyandrographolide/kg per day
- c) 0.001-0.02 mg neoandrographolide/kg per day.

23. Use of the composition, according to claims 21 and 22, characterized in that it is useful for treating autoimmune diseases.

24. Use of the composition, according to claims 21 and 22, characterized in that it is useful for treating rheumatoid arthritis.

25. Use of the composition, according to claims 21 and 22, characterized in that it is useful for treating lupus exanthematous.

AMENDED SHEET (ARTICLE 19)

26. Use of the composition, according to claims 21 and 22, characterized in that it is useful for treating multiple sclerosis.

5           27. Use of the composition, according to claims 21 and 22, characterized in that it is useful for preventing and treating Alzheimer's disease.

28. Use of the composition, according to claims  
10 21 and 22, characterized in that it is useful for treating asthma and allergies.

29. Use of the composition, according to claims 21 and 22, characterized in that it is useful for treating  
15 psoriasis.

30. Use of the composition, according to claims 21 and 22, characterized in that it is useful for treating systemic dermatomyocytis.  
20

31. Use of the composition, according to claims 21 and 22, characterized in that it is useful for treating osteoarthritis.

25           32. Use of the composition, according to claims 21 and 22, characterized in that it is useful for treating the acquired immune deficiency syndrome (AIDS).

33. Use of the composition, according to claims 21 and 22, characterized in that it is useful for treating diabetes mellitus.

5

34. Use of the composition, according to claims 21 and 22, characterized in that it is useful for treating rejection of organs and tissues in transplanted patients.

10

35. Pharmaceutical compositions, according to claim 18 CHARACTERIZED in that it can be enteral, parenteral, dermic, ocular, nasal, otic, rectal, vaginal, urethral, buccal, pharyngeal-tracheal-bronchial pharmaceutical forms, wherein the pharmaceutical composition comprises a dried extract containing a diterpenic Labdane mixture wherein each Diterpenic Labdane is present within the mixture in an amount from about 20 to about 40% w/w of Andrographolide, from about 3 to about 6% w/w of 14-Deoxyandrographolide, and from about 0.2 to about 0.8 % w/w of Neoandrographolide.

36. The pharmaceutical compositions, according to claim 35, characterized in that the diterpenic labdanes are present in an amount from about 25 to 35% w/w of Andrographolide, from about 4.5 to about 5.5% w/w of 14-Deoxyandrographolide, and from about 0.4 to about 0.8 % w/w of Neoandrographolide.

37. The pharmaceutical composition according to claim 36, wherein each diterpenic labdanes is present in an amount of 24.6% w/w of Andrographolide, 4.8% w/w of 14-Deoxyandrographolide, and 0.6% w/w of Neoandrographolide.

38. Pharmaceutical compositions, according to anyone of claims 35 to 37, characterized in that they are administered by the corresponding routes, in the following doses:

- a) 1-5 mg andrographolide/kg per day
- b) 0.02-0.5 mg 14-deoxyandrographolide/kg per day
- c) 0.001-0.02 mg neoandrographolide/kg per day.

39. Use of the pharmaceutical composition, according to claims 35 to 38, characterized in that it is useful for treating autoimmune diseases.

40. Use of the pharmaceutical composition, according to claims 35 to 38, characterized in that it is useful for treating rheumatoid arthritis.

41. Use of the pharmaceutical composition, according to claims 35 to 38, characterized in that it is useful for treating exanthematous lupus.

42. Use of the pharmaceutical composition, according to claims 35 to 38, characterized in that it is useful for treating multiple sclerosis.

5 43. Use of the pharmaceutical composition, according to claims 35 to 38, characterized in that it is useful for preventing and treating Alzheimer's disease.

44 Use of the pharmaceutical composition,  
10 according to claims 35 to 38, characterized in that it is useful for treating asthma and allergies.

45. Use of the pharmaceutical composition, according to claims 35 to 38, characterized in that it is  
15 useful for treating psoriasis.

46. Use of the pharmaceutical composition, according to claims 35 to 38, characterized in that it is useful for treating systemic dermatomyocytis.  
20

47. Use of the pharmaceutical composition, according to claims 35 to 38, characterized in that it is useful for treating osteoarthritis.

25 48. Use of the pharmaceutical composition, according to claims 35 to 38, characterized in that it is useful for treating acquired immune deficiency syndrome (AIDS).

AMENDED SHEET (ARTICLE 19)

49. Use of the pharmaceutical composition, according to claims 35 to 38, characterized in that it is useful for treating diabetes mellitus.

5

50. Use of the pharmaceutical composition, according to claims 35 to 38, characterized in that it is useful for treating the rejection of organs and tissues in transplanted patients.

10

Figure 1

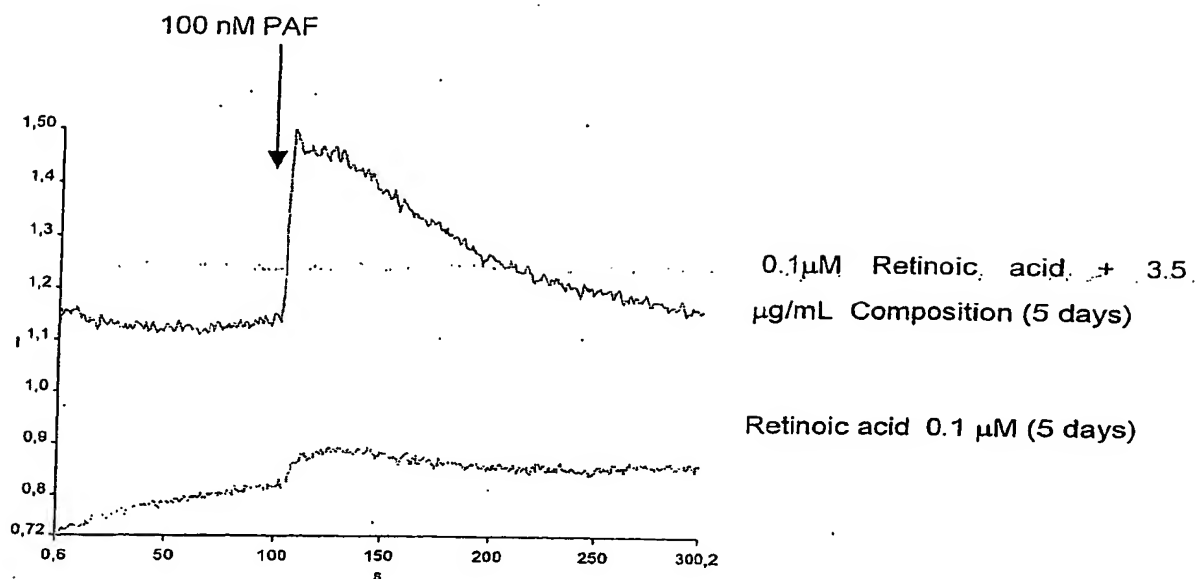


Figure 2

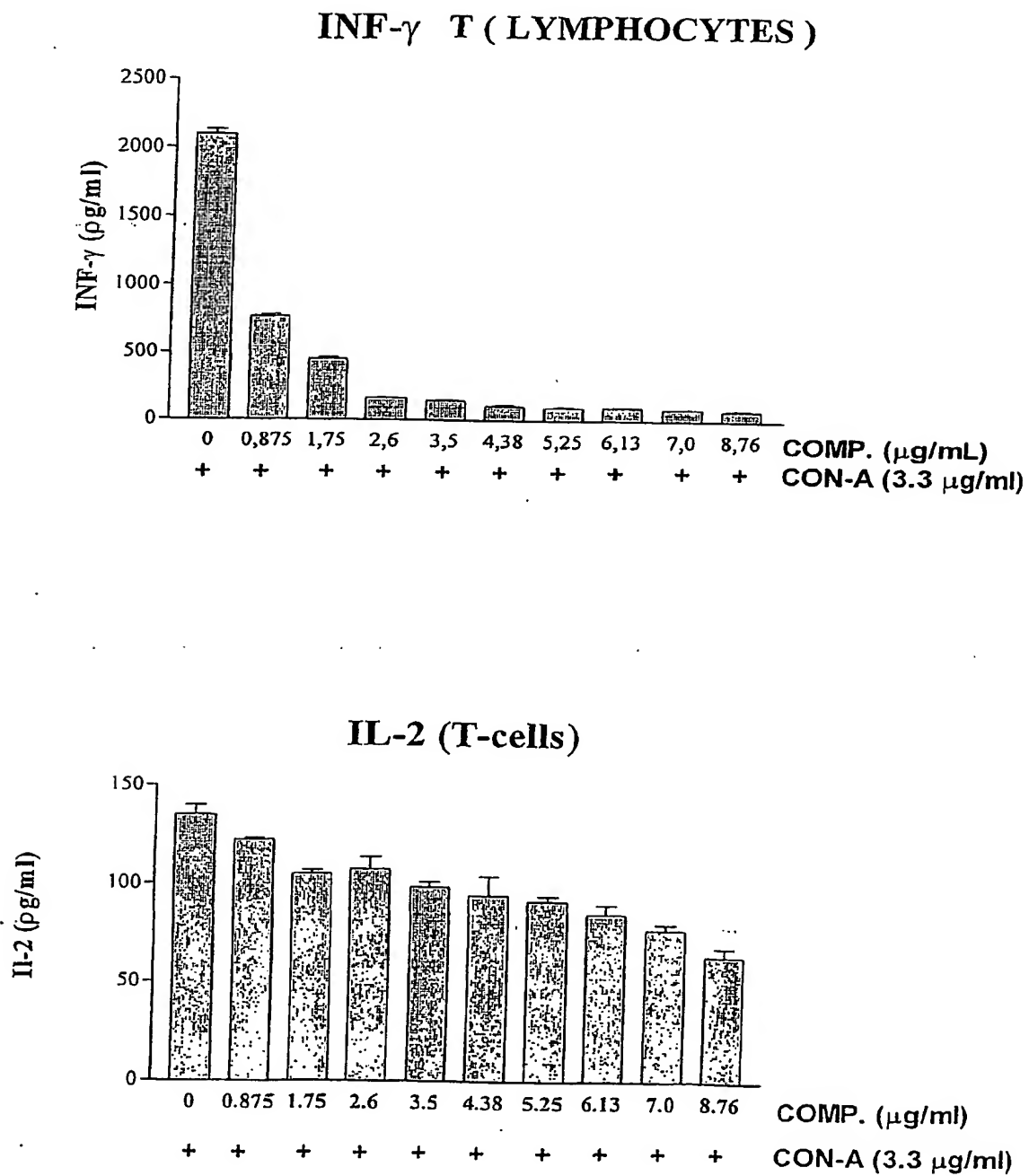
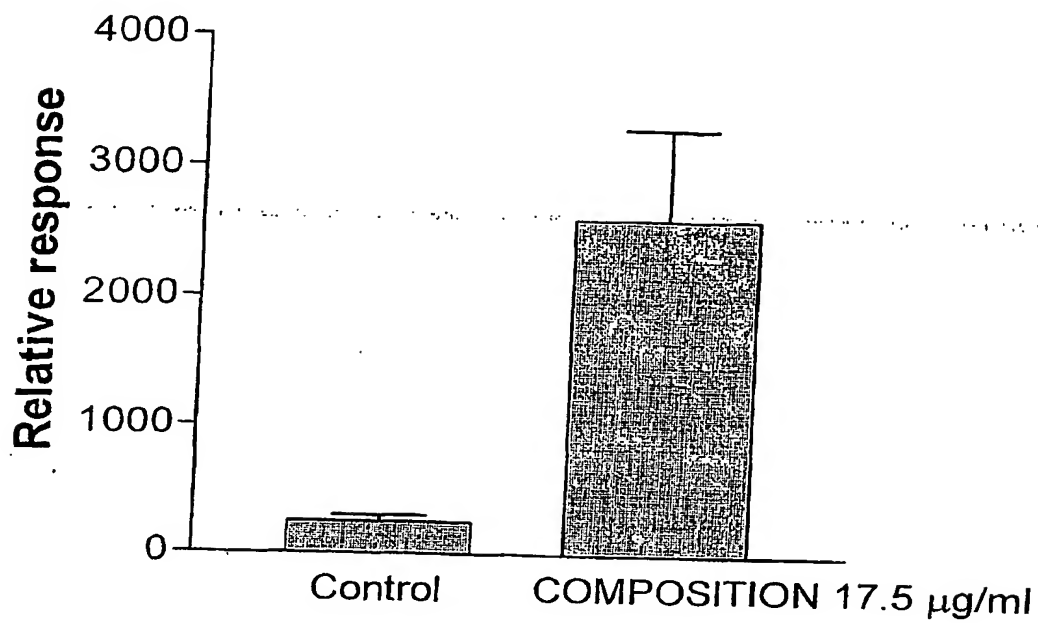
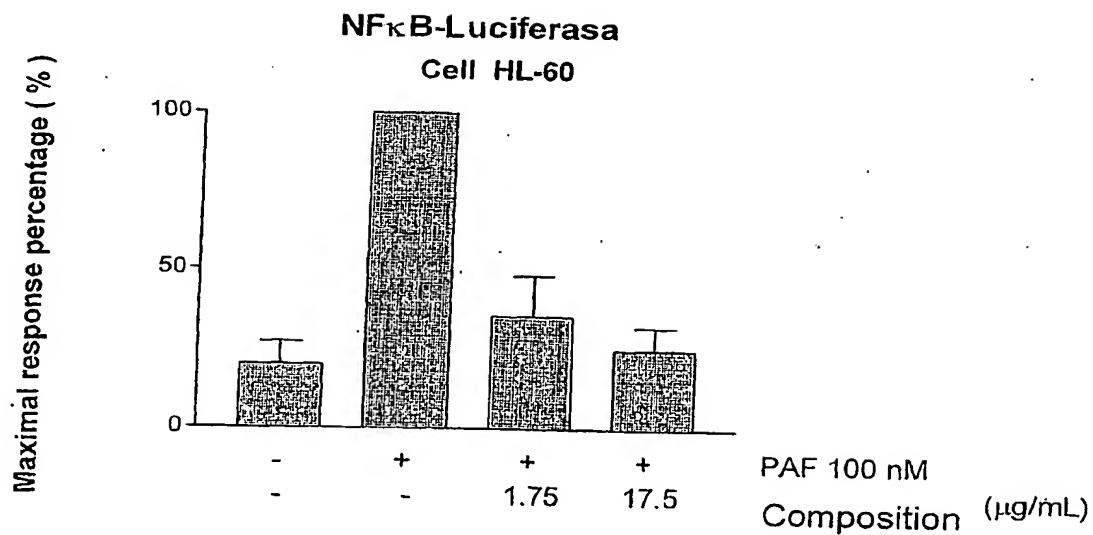
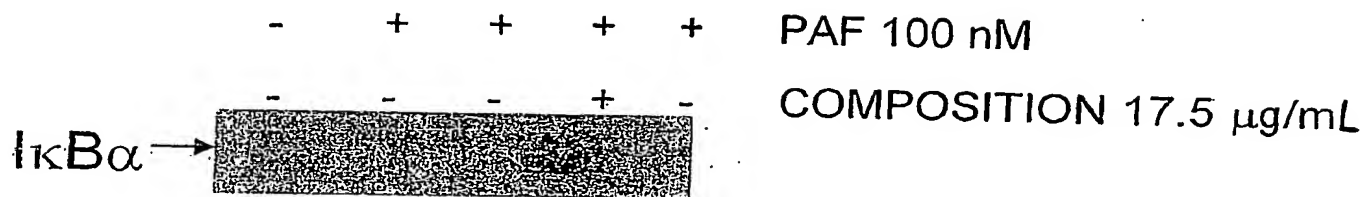


Figure 3

**PPAR $\gamma$ -Luciferase**

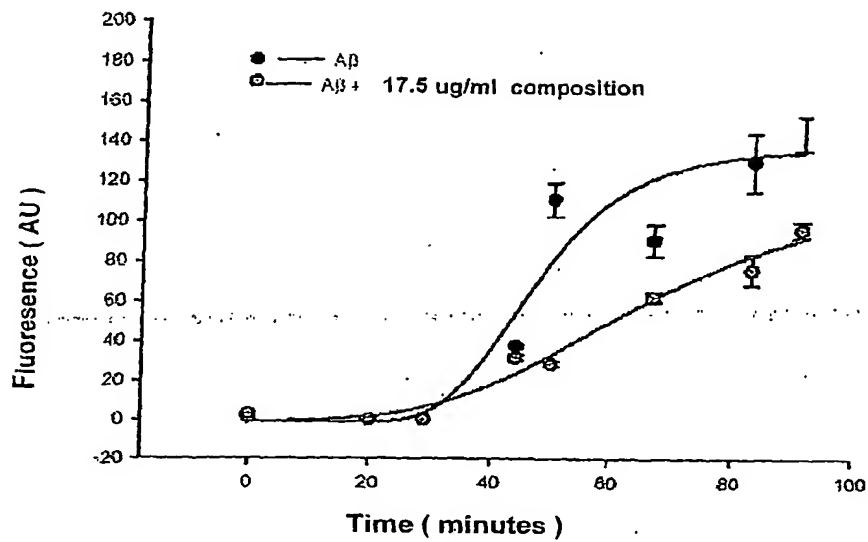
4 / 5

Figure 4

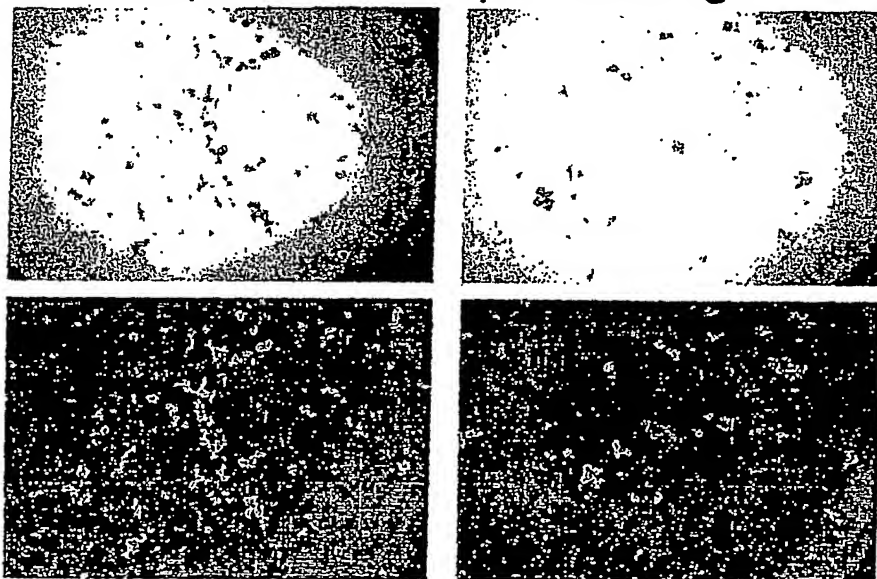


5 / 5

Figure 5



Ct Aβ 26 hrs.    Aβ + 17.5 ug/ml de la composition



# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/LY/2004/005516

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 7 A61K35/78 A61P37/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, EMBASE, WPI Data, PAJ, CHEM ABS Data

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>WO 96/17605 A (PARACELSIAN INC) 13 June 1996 (1996-06-13)</p> <p>page 6, line 7 - page 7, line 9 ----- -/--</p>	<p>1-5, 10, 15, 18-23, 28, 33, 36-40, 45, 50</p>

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
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Date of the actual completion of the international search

6 October 2004

Date of mailing of the international search report

21/10/2004

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
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Fax: (+31-70) 340-3016

Authorized officer

Friederich, M

## INTERNATIONAL SEARCH REPORT

International Application No  
PCT/LI 2004/005516

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEUNG H Y ET AL: "Determination of bioactive diterpenoids from <i>Andrographis paniculata</i> by micellar electrokinetic chromatography" JOURNAL OF CHROMATOGRAPHY A, ELSEVIER SCIENCE, NL, vol. 930, no. 1-2, 28 September 2001 (2001-09-28), pages 171-176, XP004306385 ISSN: 0021-9673 table 2	1-5,15, 16, 18-23, 33,34, 36-40, 50,51
X	HANDA S S ET AL: "Hepatoprotective activity of andrographolide from <i>Andrographis paniculata</i> against carbontetrachloride." THE INDIAN JOURNAL OF MEDICAL RESEARCH. AUG 1990, vol. 92, August 1990 (1990-08), pages 276-283, XP009037578 ISSN: 0971-5916 page 277, column 1, paragraph 2	1-5, 18-23, 36-40
X	US 2003/108628 A1 (BABISH JOHN G ET AL) 12 June 2003 (2003-06-12)  page 1, column 1, last paragraph - page 1, column 2, paragraph 1; examples 6,7,10	1-16, 18-34, 36-51
X	GUPTA P P ET AL: "Antiallergic activity of andrographolides isolated from <i>Andrographis paniculata</i> (Burm. F) wall" PHARMACEUTICAL BIOLOGY, vol. 36, no. 1, January 1998 (1998-01), pages 72-74, XP009037523 ISSN: 1388-0209 abstract	1,2,4,5, 11, 18-23, 29, 36-40,46
X	ZHANG X-F ET AL: "Antihyperglycaemic and anti-oxidant properties of <i>Andrographis paniculata</i> in normal and diabetic rats" CLINICAL AND EXPERIMENTAL PHARMACOLOGY AND PHYSIOLOGY 2000 AUSTRALIA, vol. 27, no. 5-6, 2000, pages 358-363, XP002299447 ISSN: 0305-1870 abstract	1-6,16, 18-24, 34, 36-41,51

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/EP2004/005516

## Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
  
Although claims 5-17, 24-35 and 41-52 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 2004/005516

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9617605	A	13-06-1996	AU 4376396 A	26-06-1996
			WO 9617605 A1	13-06-1996
US 2003108628	A1	12-06-2003	CA 2454171 A1	30-01-2003
			EP 1411959 A1	28-04-2004
			WO 03007975 A1	30-01-2003

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